IDL - International Digital Library Of
Science & Research
Volume 2, Issue 2, June- 2018

Available at: www.dbpublications.org

International e-Journal For Science And Research-2018

"*LIPID PEROXIDATION PRODUCTS IN FRY OF PANGASIANODON HYPOPHTHALMUS EXPOSED TO COPPER*"

Dr. S. Pratima Kumari[1] , Dasgupta Girish[2] & Kaniska Dasgupta [3].
Dept. of Zoology, Ch.Sd.St Theresa's College for Women, Eluru.
[1, 2]College Student of Chaitanya, Young Scholars
Eluru, India
[1]spratima67@yahoo.com., Ph. 7989696632

Abstract

Lipid peroxidation is a well defined mechanism of cellular damage in both animals and plants that occurs *in vivo* during disease states, stress and ageing. Lipid peroxides are unstable markers of oxidative stress, which decompose to form complex, reactive by products. It is used as an indicator of oxidative stress in cells and tissues. Measurement of malondialdehyde and 4–hydroxyl alkenes has been used as an indicator of lipid peroxidation (LPO) Pollution in aquatic environment due to various toxicants which has been reported by several investigators. The presence of such metals in biological systems in free form can significantly increase the level of oxidative stress. Reactive oxygen species (ROS) are ions or very small molecules that include free radicals, peroxides and reactive anions containing oxygen atoms or molecules that can either produce free radicals or are chemically activated by them sometimes either due to pathological conditions or other natural or manmade sources, which lead to imbalance between the ROS and anti-oxidant defense system.

Keywords: Free radicals, Oxidative stress, Decrease membrane integrity, Genotoxic effect, Oxidative damage, Tumorogenicity, Super oxide.

1. INTRODUCTION

Kappus and Sies, (1981) reported that enhanced lipid peroxidation, is a consequence of oxidation, deterioration of membrane lipids and is generally referred to as an index of oxidative stress. Harmen, (1981) stated that lipid peroxidation and free radical generation are complex and deleterious processes which are closely related to toxicity. Dormandy, (1983) stated that lipid peroxidation is caused by hydrogen peroxide (H_2O_2), hypocholorous acid (HOCl) and free radicals such as hydroxyl radical (OH), superoxide anion (O_2^-), nitric oxide (NO) and peroxynitrite ($ONOO^-$). This series of ROS initiated lipid peroxidation reactions with the production of lipid peroxyl and alkoxyl radicals which are collectively called chain propagation reactions in which oxygen free radicals may cause damage more than their initial reaction products. Studies performed in vitro suggest that exposure to oxygen radicals result in peroxidation of membrane lipids, are accompanied by damage to cellular organelles and to membrane bound enzymes (Guarneri *et al.*, 1983; Kramer *et al.*, 1984; and Kukreja *et al.*, 1988). According to Takeda *et al.* (1984), ROS not only produce peroxidation of poly-unsaturated fatty acids, causing injury to plasma membrane, leading to abnormal function and loss of membrane integrity, but also attack proteins resulting in their aggregation or fragmentation, inactivation of membrane enzymes, receptors, transport proteins, and modifying cellular antigenic properties along with damaging the nucleic acid. Halliwell and Gutteridge, (1984) reported that superoxide anions which are precursors to active free radicals have potential of reacting with biological macromolecules thereby inducing tissue damage that ultimately initiate lipid peroxidation. Oxygen free radical toxicity results in lipid peroxidation. Among the ROS, the hydroxyl radicals (OH) are

the most reactive and predominant radicals generated endogenously during aerobic metabolism. Due to the high reactivity, the radicals have a very short biological half-life. Andaya and Di Giulio, (1986) studied the acute toxicity and hematological effect of two substituted napthoquinones in channel cat fish and they found that these compounds are toxic in part due to their ability to undergo redox cycling and thereby generate reactive oxygen species. Braughler *et al.* (1987) reported that the generated hydroxyl radicals initiate the lipid peroxidation process and/or propagate the chain process via decomposition of lipid hydro peroxides. Nishimoto *et al.* (1991) opined that oxidative stress can thus lead to damage in DNA and DNA base modification, single and double strand breaks and the formation of apurinic/apyrimidinic sites, deamination, nitration and DNA-protein cross links.

Macklin and Bendich, (1987) noticed that lipid peroxidation is one of the several mechanisms by which reactive oxygen species may be toxic to cells and tissues. According to Nazerath *et al.* (1991), mitochondrial oxidative damage and membrane permeability have direct implication on nervous system leading to variety of pathological conditions. Under certain conditions, mitochondrial super oxide generation was increased or the anti-oxidant systems were depleted at which H_2O_2 production and the steady state concentration of oxygen in the mitochondrial matrix was about 5 to 10 fold higher than that in the cytosolic and nuclear spaces. Reactive oxygen species generated by mitochondria or from other sites within or outside the cell could cause damage to mitochondrial components and initiate degradation of the cell.

The hydroxyl radical is particularly unstable and reacts rapidly and non-specifically with most biological molecules which cause lipid peroxidation, DNA damage and impair calcium homoeostasis (Winston and Di Giulio, 1991; Stohs and Bagchi, (1995). Halliwell & Chirico, (1993), and Andrews, (2000) reported that oxidative stress occurs in organisms if the steady state balance of naturally occurring reactive oxygen species and anti-oxidants, capable of deactivating reactive oxygen species, is upset. According to them, the metals such as iron, copper, chromium, vanadium and cobalt are capable of redox cycling in which a single electron may be accepted or donated by the metal. This action catalyzes production of reactive radicals and reactive oxygen species. Lemaire and Livingstone, (1993) observed that the formation of ROS may also be enhanced by xenobiotics through induction of cytochrome P-450 system, or uptake of lipophilic xenobiotics into membranes resulting in disturbance of electron flow between components of the cytochrome P-450 system.

According to Stohs and Bagchi, (1995), the most important reactions are Fenton's reaction and Haber-Weiss reaction in which the hydroxyl radical is produced from reduced iron and hydrogen peroxide. Davies (1995) and Dies *et al.* (1997) stated that in general, anti-oxidant systems either prevent these reactive species from being formed or remove them before they can damage vital components of the cell. Davies, (1995) reported that the vast majority of complex life on earth requires oxygen for its existence, as oxygen is highly reactive molecule that damages living organisms by producing reactive oxygen species. Sies, (1997) and Vertunani *et al.* (2004) observed that organisms contain a complex network of anti-oxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. Girard and Boiteux, (1997) reported that it may lead to tumorogenicity. Doyotte *et al.* (1997) strongly established that toxicity caused to aquatic organisms by heavy metals particularly the transitional group is partly due to excess generations of reactive oxygen species. According to him, the health of aquatic organisms is linked to the over-production of reactive oxygen species in their tissues. Though reactive oxygen species are crucial to normal biological processes, they are potentially dangerous as observed by Zimmermann, (1998) and Toyokuni, (1999) and these are commonly referred to as pro-oxidants Mates *et al.* (1999). Lelli *et al.* (1998), and Lee & Shacter, (1999) noted that severe levels of oxidative stress can cause necrosis, ATP depletion causing the cell to simply fall apart. Fridovich, (1999) observed that hydrogen peroxide is another important reactive oxygen species contributing to oxidative stress and they are unstable and very reactive molecules physiologically whereas they are highly deleterious and cytotoxic oxidants at pathological levels.

Freidovich, (1999), Evans and Halliwell, (2001) and Fang, (2002) stated that H_2O_2 is produced through two-electron reduction of oxygen by cytochrome P-450, D-amino acid oxidase, acetyl coenzyme A-oxidase, or uric acid oxidase and oxidation of sarcosine in the pathway of glycine

metabolism. Fang, (2002), and Wu & Meninger, (2002) reported that free radicals cause several diseases such as liver cirrhosis, atherosclerosis, cardio vascular diseases, cancer, aging, arthritis and diabetes. According to them, the superoxide is generated from oxygen by multiple pathways such as NADPH oxidation by NADPH oxidase, oxidation of xanthine or hypoxanthine by xanthine oxidase and oxidation of reducing equivalents (eg. NADH, NADPH and $FADH_2$) via the mitochondrial electron transport system, anti-oxidation of monoamines (eg. Dopamine, epinephrine, and nor-epinephrine), flavins, and haemoglobin in the presence of trace amount of transition metals. One electron reduction of oxygen occurs either by cytochrome P-450 or n Nos and eNos when arginine or tetrahydrobiopterin is deficient. Most of the oxygen derived species are produced at a low level by normal aerobic metabolism and the damage they cause to cells is constantly repaired. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell including protein, lipids and DNA leading to a condition termed as oxidative stress. Toyokuni, (1999); Tsukahara,(2002) therefore stated that organisms have evolved a number of interrelated anti-oxidant mechanisms to maintain redox homeostasis. (Madeo *et al*. 1999; Fleury *et al.* 2002) observed that reactive oxygen species not only act as external triggers of apoptosis, but also play a crucial role as mediators of apoptosis. Halliwell and Gutteridge, (1999) reported that any process which leads to increased reactive oxygen species production either directly or indirectly via organic radical formation or other mechanisms can potentially result in enhancement of oxidative stress and biological damage. They assessed that at another phase, they may also damage and attack biological macro molecules, namely lipids, proteins, DNA inducing oxidation and cause membrane damage, enzyme inactivation and DNA damage. Raha and Robinson, (2000) therefore stated that the use of oxygen as a part of the process for generating metabolic energy produces reactive oxygen species. Livingstone *et al.* (2000) noticed that the ROS are continuously produced as undesirable toxic byproducts of normal metabolism from various endogenous processes and it has been estimated that about 1-3% of oxygen consumed in animal system is converted to reactive oxygen species. Nitric oxide formed during their reaction with oxygen or with superoxide, such as NO_2, N_2O_4, N_3O_4, NO_3^- and NO_2^- are very reactive. Moncada *et al.* (2001) reported that these compounds are responsible for altering the structural and functional behavior of many cellular components. It is also implicated for inflammation, cancer and other pathological conditions. Srinivas *et al*. (2006) reported that heavy metals affect multiple physiological systems such as specific biochemical processes, enzyme activity and/or membrane specific reactions. Vutukuru *et al*. (2006) reported that copper exhibits redox potential and generates lipid peroxides in fresh water fish *Esomus danricus* to cope up stress. Rhee, (2006) however, noticed that reactive oxygen species do have useful functions in cells, such as redox signaling, anti-oxidant system but the latter is not to remove oxidants entirely, but instead to keep those at an optimum level. Oxidative stress is imposed on organisms as a result of three factors: first, an increase in oxidant generation, second, a decrease in anti-oxidant protection, and third, a failure to repair oxidative damage as reported by Zhang *et al*. (2008). He also studied on the role of water borne copper on survival, growth and feed intake of Indian major carp, *Cirrhinus mrigala* (Ham). While investigating the effect of copper on growth, protein, lipid and glycogen, they noticed increased levels of lipid peroxidation products. Wang *et al*. (2009) estimated the levels of lipid peroxidation, total glutathione, catalase, glutathione-s-transferase and acetylcholine-esterase activities in gill, brain, liver and muscle on exposure to enrofloxacin EF. His results suggested that enrofloxacin EF exposure may lead to disorders like lipid peroxidation and neural dysfunction in fish. However, when reared under lower stress condition (medium density), they may cope better with EF induced stress than chronically stressed fish. Minghong *et al.* (2011) studied the oxidative stress in zebra fish embryos induced by short term exposure to bisphenol A, nonylphenol and their mixture**.** Exposure to the chemicals was found to enhance the production of hydroxyl radicals and lipid peroxidation in a concentration dependent manner. The content of total glutathione, reduced glutathione and oxidized glutathione, as well as the activity of anti-oxidant enzymes including catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione S–transferase were all significantly inhibited after exposure to BPA, NP and BPA-NP, indicating the occurrence of oxidative stress. Co-exposure to BPA-NP resulted in an additive effect on some anti-oxidant parameters. In addition, the alkaline phosphatase activity was also significantly inhibited after exposure to BPA, NP and their mixtures.

A review of literature indicates that there are only few investigations on the lipid peroxidation products and other anti-oxidant enzymes in aquatic organisms in relation to toxicants especially copper. Earlier investigations on the fry of *Phangasianodon hypophthalmus* indicate that there was tissue accumulation of copper, decrease in oxygen consumption and associated changes in different biochemical constituents with decrease in growth of the animal Therefore, an attempt has been made to study the production of lipid peroxidation products which is an indicator of oxidative stress in the fry of *Phangasianodon hypophthalmus* exposed to sub lethal concentration of copper.

2. .MATERIAL

The fish fry of *Pangasianodon hypophthalmus* were collected from the local vendors of fish market and maintained in the laboratory as described earlier. Before experimentation they were acclimatized to the laboratory conditions for 48hrs. Almost uniform sized (2.8 - 3.0 cms) active and healthy fish fry were selected for the experiment. The fish were maintained in the plastic trough of 50L capacity. For every 24 hrs the water has been renewed. The fish fry were fed by rice bran and wheat bran twice a day. But the feeding was stopped at 6 hrs before experimentation. Every day the fecal matter and the left over feed were removed by siphoning. Dead animals that did not respond to the touch of a blunt needle were removed from the troughs every day when the test solutions were renewed.

The fish fry were exposed to a sub lethal concentration of 0.0915 ppm of copper ($1/5^{th}$ of 96 hrs LC_{50}) for a period of 30 days. Parallel controls were maintained simultaneously without the metal toxicant. Test solution was prepared by dissolving copper sulphate (AR) in distilled water and appropriate amount were added to the fresh water to get the final desired concentrations described earlier. Samples were taken both from control and exposed at intervals of 24 hrs, 96 hrs, 10 days, 20 days and 30 days for the estimation of lipid per oxidation products as per the procedure given below:

3.METHODOLOGY

Lipid peroxidation products were estimated in control and exposed fry of *Pangasianodon hypophthalmus* by following the method of Hiroshi *et al*. (1979). A 10% homogenate was prepared in 1.5% potassium chloride solution. One milliliter of the homogenate was added to 2.5 ml of 20% TCA. The mixture was centrifuged at 3,500 rpm for ten min at 4^oC. The pellet obtained was dissolved in 2.5 ml of 0.05M H_2SO_4 and then 3 ml of 2M TBA (Thiobarbutric acid) was added to it. The test tubes were incubated in boiling water bath for 30 min, at 100^o C. The contents were cooled and the colour was extracted into 4 ml of n-butanol. The colour was read at 530 nm using spectrophotometer Rayleigh–UV 9200 against a blank. The results were expressed as micromoles of malondialdehyde (MDA formed /gram weight of tissue).

4. STATISTICS

The experiments on lipid per oxidation products for control and exposed fish fry were repeated 3 times and the assay was carried out in triplicate. Based on the results obtained, the mean values and standard deviation were calculated at each interval for both the control and exposed fish. The percent increase over their respective controls was calculated at each interval. For comparing the results between control and exposed fish fry, One way - ANOVA test was performed.

5. RESULTS

Table and Figure show the result of the present investigation on lipid per oxidation products formed in the tissues of *Pangasianodon hypophthalmus* fish fry on exposure to sub lethal concentrations of copper.

The above data indicate that there was an increase in levels of lipid peroxidation products from 24 hrs to 30 days in the exposed fry and a significant increase ($P < 0.05$) was observed over their respective controls at all the intervals. A maximum amount of lipid peroxidation products was observed at 30 days exposure (36.8 nanomoles of MDA/gram weight of the tissue) and the lowest was (15.89 nanomoles MDA/gram weight of tissue) which was noticed at 24 hrs exposure. However, more increase was recorded both at 96 hrs and 30 days interval. At 96 hrs and 10 days almost similar values in percent decrease was observed over their respective controls. The percent increase of total lipid peroxidation products in the exposed fry over their respective controls at 24 hrs, 48 hrs, 96hrs, 10 days, 20 days and 30 days were 15.89, 23.54, 28.82, 28.86, 30.02, and 36.85 respectively.

DISCUSSION AND CONCLUSION

The results indicate that copper induces the formation of lipid peroxidation products in the tissues of exposed *Pangasianodon hypophthalmus* fry. Several heavy metals are found to induce the production of lipid peroxidation products (LPO) in organisms.

Lushchak *et al*. (2001) investigated that oxidative stress and anti-oxidant defences in gold fish *Carassius aurtus* during anoxia and reoxygenation vary upto (44-61%) in muscle. According to them, regulation of the gold fish anti-oxidant system during anoxia may constitute a biochemical mechanism that minimizes oxidative stress following reoxygenation. Pandey *et al.* (2001) studied the effect of endosulfan on anti-oxidants of freshwater fish *Channa punctatus* and found that the endosulfan induces peroxidative damage in liver, kidney, and gill in response to which anti-oxidant were modulated.

However, when fish pre acclimatized to copper were exposed to endosulfan, protection against oxidative damage was observed only in the liver. Varo *et al*. (2007) investigated the effect of sublethal concentrations of copper sulpahate on seabream *Sparus aurata* fingerlings and found that the exposure of *Sparus aurata* fingerlings to copper sulphate induced increased lipid peroxidation. Neiva Braun *et al.* (2008) investigated on silver catfish *Rhamdia quelon* juvniles exposed to different dissolved oxygen levels found that the low dissolved oxygen levels in the water lead to lipid peroxidation, but at the same time there is an increase of superoxide dismutase activity, maintaining the oxidative equilibrium.

Various types of genes might be responsible for the production of various LPP products, to cope up toxic stress. Stressors like, toxicants induce HSP 70, mRNA, which is a general biomarker of toxicant stress De Boeck *et al*. (2003). In fish, the class of cytochrome P_{450} isozymes are responsible for the transformation of a variety of environmental contaminants including polyaromatic hydrocarbons (PAHs), planar polychlorinated biphenyls (PCBs) and aryl aminesVander Oost *et al.* (2003). In fact, metabolism and carcinogenesis studies have shown CYP1B gene to be a critical and necessary enzyme producer involved in the oxidation of chemical toxicants. Godard *et al.* (2000) find out that the main function of COI gene is to transfer electrons from cytochrome - c to oxygen in mitochondrial based electron transport chain and to generate ATP. Besides, COI may also function indirectly as an anti-oxidant by either preventing the dawdling of electron flow Benzi *et al.* (1992) or by uncoupling electron transport from proton transfer Amit *et al.* 2010).

Joycelyn *et al.* (2010) noticed a peculiar pattern of lipid peroxidation of which MDA levels, indicative of oxidative stress is highly observed in the spleen followed by the liver, and the gills. Arabi *et.al*. (2005) indicated that the mercury and copper act as genotoxic pollutants, which alter the DNA integrity by including the single and double stranded DNA breaks in the gill cell nuclei, depletion of GSH content and inhibition of anti-oxidant enzyme GST resulting in the propagation of LPO and DNA damage.

Similarly in the present experiment also copper might be acting on the sensitive fish fry *Pangasianodon hypophthalmus* and might have shown decrease in membrane integrity and GSH content or genotoxic effect which result in the alternation of DNA integrity or inhibition of anti-oxidant enzyme GST resolution in the propagation of LPO and DNA damage. In the 24 hrs exposure there was minimum increase in the LPP. In 48 hrs of exposure there is more increase in LPO suddenly. It was studied that the reason may be activation of different genes to produce different types of anti-oxidant products. In the 96 hrs exposure a significant difference in the amount of LPP was seen i.e., 28.82. But almost the same difference continued even in the 10^{th} day of exposure i.e., 28.86. There might be some defense mechanism to cope up stress due to toxicity or the fish would cope with proteotoxicity by the induction of HSPs, mRNA levels, which are able to repair partly denatured proteins on 10^{th} day. The same observation was reported by Hallare (2004) while studying the development, toxicity stress and protein responses in zebra fish embryos after exposure to diclofenac and its solvents, DMSO.

The results indicate that copper induces formation of lipid per oxidation products in the tissues *Pangasianodon hypophthalmus.* Or on the 10^{th} day there was not much of LPP compared to 96 hrs. Here more of carbohydrates and moderately proteins and then fats might be used to cope up stressful conditions which can be clearly seen in biochemical constituent experiment earlier.

Mausumi *et al*. (2009) studied that the growth, protein, glycogen, lipid levels were increased in the control fish of *Cirrhinus mrigala*, (Hamilton) along with daily weight gain. This trend has further increased with the advancement of the

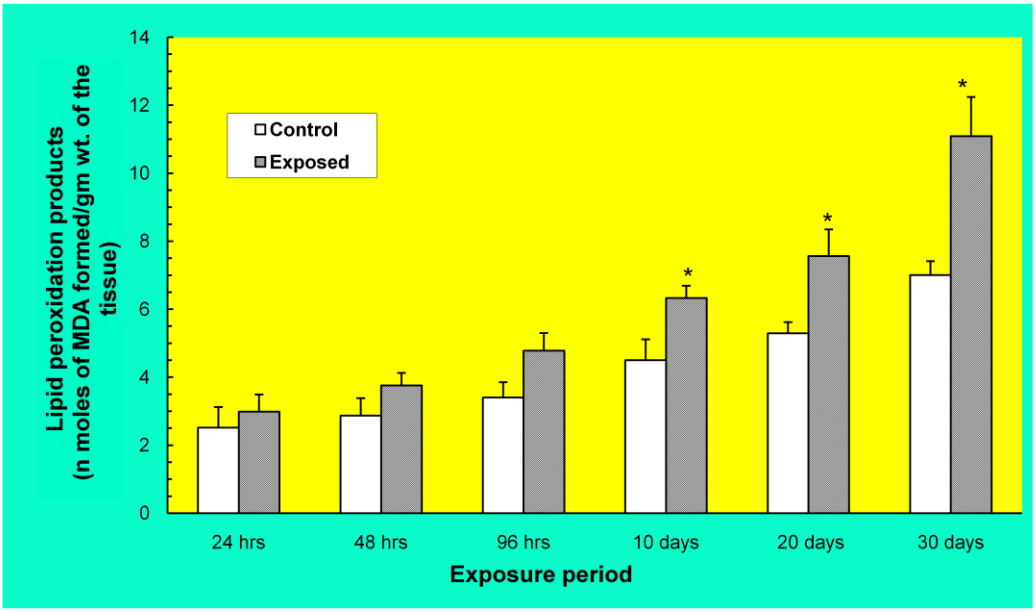
end of the 30 days experimental period. Where as a treatment with 1/5 of the LC_{50} value of copper showed that there is decrease in growth and the levels of essential elements while the lipid peroxidase level increased after 30 days.

In the present investigation, the effect of copper on *Pangasianodon hypophthalmus* fish fry reveals the formation of lipid peroxidation. Increase values in lipid peroxidation products confirm the oxidative stress due to toxic metal accumulation. This might be one of the possible reasons in reduction of all other physiological and biological parameters such as growth, total biochemical constituents and oxygen consumption experiments done earlier. The interaction of metal toxicants in inhibiting normal biochemical, physiological, and enzyme activity may be the reason behind the disturbances in biochemical and physiological metabolic pathway.

With the increase in exposure time of $1/5^{th}$ copper sulphate we see a gradual increase in the amount of lipid peroxidation. In 24 hrs because the time of exposure was very less, we find that there is minimum production of LPP which can be clearly seen by the percent difference value with control i.e., 15.89. At 96 hrs there was a drastic change in LPP value which can be attributed to the animal might have used its optimum capacity to bear the toxic effect. Later almost the same percent difference with negligible increase continued in the 10^{th} day, later on the 20^{th} day also there was not much increase which suggests that from 96 hrs to 20^{th} day the fish might have used other compensatory mechanisms to cope up toxic stress. It may be assumed that fish might be using biochemical constituents at this time. But on 30^{th} day we find that LPO was produced to its maximum at the same time the utilization of fats was also more which was studied earlier. This shows more of metal might have accumulated on 30 days exposure which require more of deleterious and bearer chemicals to nullify the effect of copper. The use of lipid per oxidation as a parametric indicator of stress in this present experiment is the first of its kind using fry of *Pangasianodon hypophthalmus.*

Copper was found to be highly toxic to the fish and induced significant declines ($p < 0.05$) in all of the biochemical profiles studied earlier demonstrating a linear and positive correlation with both the concentration and duration of exposure to sub lethal copper concentration. But in case of lipid peroxidation products reverse results were observed, where the amount of LPO was more in exposed animals when compared to controls. In *Pangasianodon hypophthalmus*, LPO levels can be employed as biomarkers of oxidative stress in aquatic environments contaminated with copper.

Lipid Peroxidation



A significant increase (P<0.05) in the exposed PL from 24 hrs onwards. i.e 15.89% at 24 hrs to 36.85% at 30 days

LPP in P.hypophthalmus fry exposed to sublethal copper. Vertical lines represents standard deviation. Significantly different from their respective controls at P<0.05

Alternations in the amount of Lipid peroxides of *Pangasianodon hypoophthalmus* exposed to 0.09156 ppm of copper. Each value represents the mean ± standard deviation. The values in the parentheses represent percent decrease over their respective controls in nano moles MDA/gm wt. weight of the tissue *significantly different from their respective controls at P < 0.05.

Groups	Exposure period					
	24 hrs	48 hrs	96 hrs	10 Days	20 Days	30 Days
Control (Cal/mg)	2.514 ± 0.606	2.874 ± 0.509	3.400 ± 0.453	4.503 ± 0.610	5.29 ± 0.324	7.003 ± 0.405

Exposed (Cal/mg)	2.989 ± 0.500* (15.89)	3.759 ± 0.368* (23.54)	4.777 ± 0.521* (28.82)	6.33 ± 0.355* (28.86)	7.56 ± 0.784* (30.02)	11.09 ± 1.151* (36.85)

ONE WAY ANOVA

Source of Variation	Degrees of freedom	Sum of squares	Mean Square	F Value
Treatments (between columns)	11	614.4	55.85	159.7
Residuals (with in Columns)	96	33.58	0.3498	
Total	107	648.0		

1. References

2. Andaya, A. and Richard, T. Di Giulio., 1986. Acute toxicities and haematological effects of two substituted naphthoquinones in channel catfish. *Archies of Environ. Conta. and Toxicol*. 16(2): 233-238.
3. Andrews, G.K., 2000. Regulation of metallothionein gene by oxidative stress and metal ions. *Biochemical Pharmacology*, 59: 95-104.
4. Arabi, M. and Alaeddini, M.A., 2005. Metal-ion-mediated oxidative stress in the gill homogenate of rainbow trout *Oncorhynchus mykiss*. Antioxidant potential of manganese, selenium, and albumin. *Biomedi. and Life Sci., Biolo. Trace Ele. Rese*., 108(1-3): 155-168.
5. Benzi, G., Pastoris, O., Marzatico, R.F., Curti, D., 1992. The mitochondrial electron transfer alteration as a factor involved in the ageing brain. *Neurobiol. Ageing* 13: 361-368.
6. Braughler, J.M., Chase, R.L and Pregenzer,J.F., 1987. Oxidation of ferrous iron during peroxidation of lipid substances. *Bio-Chem. Bio-Phys. Acta.*, 17(3): 457-64.
7. Davies, K.J., 1995. Oxidative stress the paradox of aerobic life. *Biochem. Soc. Symp.*, 61: 1-31.
8. De Boeck, G., De Wachter, B., Vlaeminck, A., Blust, R., 2003. Effect of cortisol treatment and/or sub lethal copper exposure on copper uptake and heat shock

protein levels in common carp, *Cyprinus carpio*. *Environ. Toxicol. Chem*., 22: 1122-1126.

9. Dies, S.A., Wooster, G.A., Bustos, P.S., Bowser, P.R., Babish, J.G., 1997. Multiple route and dose pharmaco kinetics of enrofloxacin. *J. Vet. Pharmacol. Ther.*, 20: 111-123.
10. Dormandy, T.L., 1983. An approach to free radical. *Lancet*, 29: 1010-1014.
11. Doyotte, A., Cossue, C., Jacquin, M.C., Babut, M. and Vesseur, P., 1997. Antioxidant enzymes glutathionate and lipid peroxidation as relevant biomarkers of experimental and field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*. *Aqua. Toxicol*., 39: 93-110.
12. Evans, P. and Halliwell, B., 2001. Micronutrients: Oxidant/ antioxidant status. *Br. J. Nutr*., 85(2): 67-74.
13. Fang, Y.Z., 2002. Free radicals and nutrition. Theory and application of free radical biology. *Beijing Scientific Press*: 647.
14. Fleury, C., Mignotte, B. and Vayssiere, J.L., 2002. Mitochondrial reactive oxygen species in cell death signalling. *Biochem*., 84: 131-141.
15. Freidovich, I., 1999. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? *Ann. N.Y. Acad. Sci*., 893: 13-18.
16. Girard, P.M. and Boiteux, S., 1997. Repair of oxidized DNA bases in the yeast, *Saccharomyces cerevisiae*. *Bio. Chemic.*, 79: 559-566.
17. Godard, C.A.J., Leaver, M.J., Said, M.R., Dickerson, R.L., George, S., Stegeman, J.J., 2000. Identification of cytochrome p450 IB-like sequences in two teleost fish species *Stenotomus chrysops* and *Pleuronectes platessa* and in Cetacean *Stenella coeruleoalba*. *Mar. Environ. Res*., 50: 7-10.
18. Guarnieri, C., Muscari, C., Ceconic, C., Flamigni, F and Calderera, C.M., 1983. Effect of superoxide generation of rat heart mitochondrial pyruvate utilization. *J. Mol. Cell Cardiol.* 15: 859-862.
19. Hallare, A.K., Kohler, H.R., Triebskorn, R., 2004. Development toxicity and stress protein responses in zebra fish embryos after exposure to diclofenac and its solvents, DMSO. *Chemosphere*, 56: 659-666.
20. Halliweel, B. and Gutteridge, J.M., 1984. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. *Lancet.* 23-1 (8391): 1396-7.
21. Halliwell, B. and Chirico, S., 1993. Lipid peroxidation its mechanism, measurement and significance. *American J. of Clini. Nutri*., 57: 715-725.
22. Halliwell, B., Gutteridge, J.M.C., 1999. Free radicals in biology and medicine, Third Edition, *Oxford University Press Inc.* New York, p. 661.
23. Harmen, D., 1981. Free radicals. *Proc. Natl. Acad. Sci*., USA. 78: 7124.
24. Kappus, H. and Sies, H., 1981. Toxic drug effects associated with oxygen metabolism: redox cycling and lipid peroxidation. *Experientia*., 37: 1223-1241.
25. Kramer, J.H., Tong Mark, I. and Weglicki, W.B., 1984. Differential

sensitivity of canine cardiac sarcolemma and microsomal enzymes to inhibition by free radical induced lipid peroxidation. *Circ. Res*., 55: 120-452.

26. Kukreja, B.C., Okabe, E., Schrier, G.M. and Hebb, M.L., 1988. Oxygen radical mediated lipid peroxidation and inhibition of Ca^{2+} ATPase activity of sarcoplasmic reticulum. *Arch. Biochem. Biophys*., 261: 447-457.
27. Lee, Y.J. and Shacter, E., 1999. Oxidative stress inhibits apoptosis in human lymphoma cells. *J.Biol. Chem.*, 274(28): 19792-19798.
28. Lelli, J.L., Becks, L.L., Dabrowska, M.I. and Hinshaw, D.B., 1998. ATP concerts necrosis to apoptosis in oxidant-injured endothelial cells. *Free Radic. Biol. Med*., 25(6): 694-702.
29. Lemaire, P. and Livingstone, D.R., 1993. Pro-oxidant/antioxidant processes and organic xenobiotic interactions in marine organisms, in particular the founder, *Platichthys flesus* and the mussel, *Mytilus edulis*. *Trends comp. Biochem. Physiol*., 1: 1119-1150.
30. Livingstone, L., Cecchini, S., Bertini, S., Cognetti, V.A.M., Soldani, G., Mengozzi, G., 2000. Pharmacokinetics of enrofloxacin in the sea bass *Dicentrarchus labrax*. *Aquacult*., 182: 49-59.
31. Lushcha, V.I., Lushcha, L.P., Lushchak, A.A., Mota and Marcelo H.L., 2001. Oxidative stress and anti-oxidant defences in Gold fish *Carassius auratus* during anoxia and reoxygenation. *Am. J. Physiol., Regul. Integr. Comp. Physiol*. 280: 100-107.
32. Maclin, L.J. and Bendich, A., 1987. Free radical tissue damage. Protective role of antioxidant nutrients. *FASEB,* 1: 441-445.
33. Madeo, F., Frohlich, E., Ligr, M., Sigrist, S.J., Wolf, D.H. and Frohlich, K.V., 1999. Oxygen stress: a regulator of apoptosis in yeast. *J. cell Biol*., 145: 757-767.
34. Mates, J.M., Perez-Gomez, C. and de Castro, I.N., 1999. Antioxidant enzymes and human diseases. *Clin. Biochem*. 32**:** 595-603.
35. Mausumi, M., Adhikari, S., Mohanty, P., Sarangi, N., 2009. Role of water borne copper on survival, growth and feed intake of Indian major carp, *Cirrhinus mrigala*, (Hamilton). *Bull. Environ. Contam. Toxicol*., 82: 559–563.
36. Mingyong, W., Jiangfei, C., Kuanfei, L., Yuanhong, C., Wei, H., Robert, L.T., Changjian, H., Qiaoxiang, D., 2011. Chronic zebrafish PFOS exposure alters sex ratio and maternal related effects in F1 offspring. *Environ. Toxico. and Chem*., 30(9): 2073–2080.
37. Moncada, S., Palmer, R.M.J. and Higgs, E.A., 2001. Nitric oxide: *Physio. pathophysio. and pharmaco*., 43(1): 109-110.
38. Nazareth, W., Nasser, Y. and Crompton, M., 1991. Inhibition of anoxia induced injury in heart myocytes by cyclosporine. *A.J. Mol. Cell. Cardiol*., 23: 1351-1354.
39. Neiva Braun, Ronaldo Lima De Alima, Franchesco Dalla Flora, Maicon Eder Lano, Liliane De Freitas Bauermann, Vania Lucia Lora and Bernardo Baldisserotto, 2008. Lipid peroxidation and superoxide dismutase activity in silver cat fish *Rhamdia quelen* juveniles exposed to different dissolved oxygen levels. *Ciencia. Ani. Bras*., 3: 811-814.

40. Nishimoto, M., Roubal, W.T., Stein, J.E. and Varanasi, U., 1991. Oxidative DNA damage in tissues of English sole, *Parophrys vetulus* exposed to nitrofurantoin. *Chem. Biol. Interact*., 80: 317-326.
41. Pandey, S., Ahmed, I., Parvez, S., Bin-Haque, R and Raisuddin, S., 2001. Effect of endosulfan on antioxidants of fresh water fish *Channa punctatus.* Protection against lipid peroxidation in liver by copper pre exposure. *Arch, Environ, Contam. Toxicol*., 41(3): 345-352.
42. Raha. S and Robinson, B., 2000. Mitochondria, oxygen free radical's disease and aging. *Trends Biochem. Sci*., 25(10): 502-508.
43. Rhee, S.G., 2006. Cell signaling, H_2O_2, a necessary evil for cell. *Signalling science*, 312(5782): 1882-1883.
44. Sies, H., 1997. Oxidative stress: oxidants and antioxidants. *Exp. Physiol.,* 82(2) 291-295.
45. Srinivas, S.V., Suma, C., Radha, M.K., Venkateswara, R.J. and Y., 2006. Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish, *Esomus. Biomedical and Life Sci. Fish Physio. and Biochem*., 32(3): 221-229.
46. Stohs, s. and Bagchi, d., 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med*., 18(2): 321-36.
47. Takeda, K., Shimada, Y., Amano, M., Sakal, T., Okoda, T. and Yoshiya, I., 1984. Plasma lipid peroxides and alpha-tocopheral in critically ill patients. *J. Crit. Care Med.,* 12: 957-959.
48. Toyokuni, S., 1999. Reactive oxygen species-induced molecular damage and its application in pathology. *Pathol. Int.*, 49: 91-102.
49. Toyokuni, S., 1999. Reactive oxygen species-induced molecular damage and its application in pathology. *Pathol. Int.*, 49: 91-102.
50. Tsukahara, H., 2002. Pathophysiological roles of nitric oxide in inflammatory diseases. In: *pediatric asthma and other allergic diseases*. (Eds.) A. Morikawa. Gunma: JOMO NEWS PAPER, 145-152.
51. Vander Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol*., 13: 57-149.
52. Varo, I., Navarro, J.C., Nunes, B., Guilhermino, L., 2007. Effects of dichlorvos aquaculture treatments on selected biomarkers of gilthead sea bream *Sparus aurata* (*L*) fingerlings. *Aquacult.* 266: 87-96.
53. Vertuani, S., Anguasti, a. and Manfredini, S., 2004. The antioxidants and pro-anti-oxidants network an overview. *Curr.Pharm. Des*., 10(14): 1677-94.
54. Vutukuru, S.S., Suma,Ch., Madhavi, K.R., Venkateswara Rao, J and Anjaneyulu, Y., 2006. Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the fresh water teleost fish, *Esomus danricus*. *Fish Physiol*, *Biochem*., 32(3): 221-229
55. Wang, W., Zhou, J., Wang, P., Tian, T., Zheng, Y., Liu, Y., Mai, W. and Wang, A., 2009. Oxidative stress, DNA

damage and antioxidant enzyme gene expression in the Pacific white shrimp, *Litopenaeus vannamei* when exposed to acute pH stress. *Comp. Biochem. Physiol. Cyto. Toxicol. Pharmacol.,* 150(4): 428-435.

56. Winston, G.W. and Di Giulio, R.T., 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquat. Toxicol*., 19: 137-161.
57. Wu, G. and Meninger, C.J., 2002. Regulation of nitric oxide synthesis by dietary factors. *Annu. Rev. Nutr*., 22: 61.
58. Zang, X., Yang, F., Zjang, X., Xu, Y., Liao, T., Song, S. and Wang, J., 2008. Induction of hepatic enzymes and oxidative stress in Chinese rare minnow *Gobio cyprisrarus* exposed to water borne hexabromocyclododecane (HBCDD). *Aquat. Toxicol.*, 86: 4-11.
59. Zimmerman, J.J., 1998. Redox/radical repertoire rapport: Pathophysiology and therapeutics. *Acta. Anaesthesiol. Scand.,* 42: 1-3.